BBA Report

BBA 21301

Enhancement of *Bacillus cereus* spore lytic enzyme by a heat-labile non-dialyzable factor in spore extracts

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(Received December 29th, 1971)

SUMMARY

A heat-labile, non-dialyzable factor has been found in extracts of *Bacillus cereus* T spores which enhances the murein-solubilizing activity of the spore lytic enzyme apparently by a direct effect on the enzyme.

The spore lytic enzyme of *Bacillus cereus* described by Gould and coworkers[1,2] appears to be a key enzyme in germination, hydrolyzing the glycosidic bonds in the murein of the spore cortex. Although the data thus far indicate that the enzyme is activated by virtue of its release from a bound form[3], our laboratory has made an observation which indicates that the mechanism of activation may be more involved. This communication reports the presence in extracts of spores of *B. cereus* T of a heat-labile, non-dialyzable factor which enhances the activity of the spore lytic enzyme apparently by a direct effect on the enzyme.

The presence of the enhancing factor was demonstrated after sequential $(NH_4)_2SO_4$ fractionation of sonically prepared *B. cereus* T spore extracts[4] and subsequent assay of the fractions for spore lytic enzyme activity. Spore lytic enzyme activity was present only in those fractions precipitated at 20–40% and 40–60% saturation with $(NH_4)_2SO_4$ as evidenced by the decrease in absorbance of sensitized spores (see legend to Fig. 1). Although those fractions obtained at 60–80% and 80–100% saturation were devoid of lytic activity, when they were added to the spore lytic enzyme-containing fractions enhancement of lytic activity was observed. Dialysis of the 60–80% and 80–100% fractions overnight against demineralized water at 2° (routine procedure for all fractions) resulted in no loss of enhancing activity; boiling for 10 min completely inactivated them. The subsequent experiments were conducted utilizing the dialyzed 20–40% (spore lytic enzyme) and 80–100% (enhancing factor) fractions to ensure complete separation of spore lytic enzyme from enhancing factor activity.

In Fig. 1 it can be seen that the addition of enhancing factor to spore lytic enzyme at time zero resulted in an increase in the rate of lytic activity as well as a decrease in the lag. Moreover, when spore lytic enzyme was preincubated with enhancing factor for 30 min at 30° prior to their addition to sensitized spores an additional decrease in the lag occurred, but no further rate increase was observed. In a similar experiment in which enhancing factor was preincubated with sensitized spores rather than spore lytic enzyme, the enhancement of spore lytic enzyme activity subsequently obtained was identical to that observed without preincubation. These data suggest that enhancing factor works directly on spore lytic enzyme.

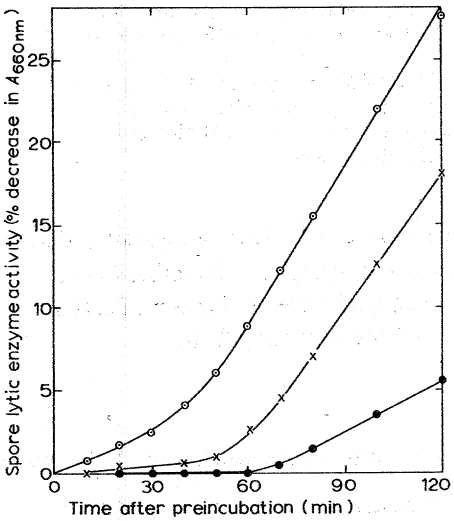

Fig. 1. Effect of preincubation of spore lytic enzyme with enhancing factor upon the decrease in absorbance of *B. cereus* T-sensitized spores. Assays were performed essentially as described by Gould *et al.*[3] with complete reaction mixture at zero time containing: sensitized spores, diluted to give a starting absorbance of approximately 0.40 at 660 nm; NaCl, 0.1 M; phosphate buffer, pH 7.0, 0.02 M; 0.1 ml of dialyzed respective fractions obtained by $(NH_4)_2SO_4$ fractionation of spore extract; and demineralized water to 3.0 ml. A decrease in the absorbance was taken as a measure of spore lytic enzyme action. ⊙, spore lytic enzyme (20–40% fraction) was preincubated with enhancing factor (80–100% fraction) for 30 min at 30°; x, enhancing factor and spore lytic enzyme were held separately for 30 min at 30° and combined at time zero; ●, spore lytic enzyme was held for 30 min at 30°. Buffer and NaCl were present during the 30-min period so that at time zero only sensitized spores were added.

To determine the effect of enhancing factor on spore lytic enzyme by means other than decrease in absorbance of sensitized spores, spore integuments were utilized as substrate, and solubilization of mucopeptide as evidenced by appearance of reducing groups was used as a measure of spore lytic enzyme activity[2]. Table I shows that solubilization of mucopeptide by spore lytic enzyme was increased 2-fold in the presence of enhancing factor, while enhancing factor itself caused no solubilization.

Finally, enhancement proceeded to the same degree in the presence or absence of NADPH, ruling out reduced pyridine nucleotide mediated activation by the disulfide reductase described by Blankenship[5]. Also incubation of spore lytic enzyme with enhancing factor for 1 h at 35° and pH 7.0 did not cause appearance of terminal amino

TABLE I

SOLUBILIZATION OF *B. cereus* T SPORE INTEGUMENTS BY SPORE LYTIC ENZYME; EFFECT OF ENHANCING FACTOR

	nmoles reducing sugar solubilized★
Integuments★★ + spore lytic enzyme	54
Integuments + enhancing factor	0
Integuments + spore lytic enzyme + enhancing factor	119

★Reaction mixtures were essentially those described for "complete reaction mixtures" in legend to Fig. 1 except that in place of sensitized spores, 1.0 mg of spore integuments was utilized as substrate. Reactions were carried out for 1 h at 35°. The integuments were then sedimented 34 000 × *g* for 30 min and the supernatant fractions assayed for reducing groups by the method of Park and Johnson[7] using glucose as the standard and the ferric ammonium sulfate reagent of Horvath and Knehr[8].

★★Integuments were prepared as described by Warth *et al.*[9] with the exception that spores were ruptured in a Mini-Mill (Gifford-Wood Co., Hudson, N.Y.) – 900 mg spores, 30 ml 0.05 M phosphate buffer, pH 7.5, 50 g No. 380-5005 Superbrite glass beads (Minnesota Mining and Manufacturing Co., St. Paul, Minn.) – for 30 min with a slit setting of 20.

groups as measured by coupling with 2,4-fluorodinitrobenzene[6], thereby suggesting that the action of enhancing factor upon spore lytic enzyme is highly specific rather than a manifestation of a general proteolytic activity. Elucidation of the mechanism of action awaits purification of both the spore lytic enzyme and the enhancing factor.

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